THE MALARIA PARASITE IN THE MOSQUITO.

THE EFFECTS OF LOW TEMPERATURE AND OTHER FACTORS ON ITS DEVELOPMENT.

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In accounting for the geographic distribution of malarial fever early investigators realized that temperature was an important factor. Following the incrimination of the mosquito as the carrier of malarial parasites, writers on the epidemiology of the disease were of the opinion that thermic conditions were in part responsible for the infection. The influence of temperature on the developmental cycle of the malarial parasites was fully recognized by the Italian workers, and their first experiments to establish the insect rôle took this important correlation into consideration.

Historical.

Bastianelli and Bignami (1899)[1] in experiments with 50 specimens of *Anopheles maculipennis* and *Plasmodium falciparum* attempted to transmit the infection at a low room temperature, 18° to 22° C. During a period of 20 days these mosquitoes when dissected showed only forms of early development. When they were removed to a temperature of 30° C. for two days or more, however, sporozoites developed. "Evidently at a temperature of 18° to 22° C. the life cycle of the parasite (*Plasmodium falciparum*) is completed very slowly."

Marchiafava and Bignami (1900)[2] noticed that the temperature exerted a certain influence upon the time necessary for the completion of the cycle. At a temperature of 20° to 22° C. the development was found to be much slower in estivo-autumnal malaria and it appeared from their observations that development did not occur at all at 14° to 15° C.

Giles (1902)[3] writes relative to geographic distribution, "It is a long-established fact that the northern limit of malaria corresponds roughly with the summer maximum isotherm of 76° F., or, according to Hirsch, to a mean summer temperature of 15° to 16° C. (60° F.), which is much the same thing. Recent Italian researches show that the development of the hæmosporidia within the mosquito can not take place at a lower temperature than 20° C. (68° F.), or at a higher temperature than 30° C. (86° F.), and in the existence of this upper limit we find an explanation of the fact that the hot dry weather in northern India, where for months together the temperature falls

[1] Bastianelli, G. and Bignami, A. (1899). Sullo sviluppo dei parasito della terzana nell' *Anopheles claviger*. Bull. d. R. Accad. Med. di Roma, Anno 25 Fasc. 3, Apr. 19. Quoted from Craig. The malarial fevers. 1909. Wm. Wood & Co., New York, p. 88.

[2] Marchiafava, E., and Bignami, A. (1900). Malaria. Twentieth century practice. Wm. Wood & Co., New York, p. 88.

[3] Giles, G. M. (1902). A handbook of the gnats or mosquitoes. Second edition. John Bales Sons & Danielsson, London, pp. 161-162.

rarely as low as this, is, in spite of the unbearable heat, by far the healthiest season of the year, and during it, primary cases of malaria are practically unknown."

Braun (1906)[1] states that the development of the sporonts in mosquitoes is dependent on the atmospheric temperature and the species of parasite; that the *Plasmodium* of the malignant tertian completes its development within eight days at a temperature of 28° to 30° C.; below 18° C. the development ceases.

Stephens (1908)[2] quotes Grassi by stating that the sporogonic developmental cycle requires, in the case of the malignant tertian parasite, an optimum temperature of about 27° C. and ceases at such lower temperatures as 15.5° to 17.5° C. In the case of the simple tertian, however, provided a suitable initial temperature has been maintained, development will still go on at temperatures as low as 12° or 9° C. But the appearance of sporozoites is then delayed for 21 days. Further, the lowest temperature at which the simple tertian parasite will develop in the mosquito is 20° to 22° C. and in the case of the quartan parasite, 16.5° C.

Craig (1909)[3] writes: "We know that the malarial plasmodia will undergo development only in stomachs of mosquitoes living under proper conditions as regards temperature, it having been proved by Jansco that the oocysts develop best at a temperature of between 20° and 30° C., while if the temperature be lower than 16° C. the organisms perish."

Ross (1910)[4] in accounting for the number of ingested sexual parasites which reach maturity and develop protospores (sporozoites) asserts that it depends among other things upon temperature. He agrees with Jansco, who finds that the zygotes develop best at 24° to 30° C., temperatures above and below these limits retarding the process; and that they die if the mosquito is kept constantly below 16° C. after feeding. On the other hand, Ross finds that they often continue to grow if the mosquito carrier is subjected merely to an intermittent low temperature.

Howard, Dyar, and Knab (1912)[5] discussing the incubation period of the sporogonic cycle as influenced by temperature, state that the most favorable temperature lies between 22° and 28° C. and beyond these conditions the development of the parasite goes forward more slowly, "and there are observations which show a period of more than 50 days."

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[1] Braun, M. (1906). The animal parasites of man. Third edition. Bale Sons & Danielsson, London, p. 98.

[2] Stephens, I. W. W. (1908). Malaria in its relation to the mosquito. Nothnagel's Encyclopedia of Practical Medicine. Saunders Co., Philadelphia and London, pp. 129–130.

[3] Craig, C. F. (1909). The malarial fevers. Wm. Wood & Co., New York, p. 109.

[4] Ross, R. (1910). The prevention of malaria. John Murray, London, pp. 87–88.

[5] Howard, L. O., Dyar, H. G., and Knab, F. (1912). The mosquitoes of North and Central America. The Carnegie Institution of Washington, Vol. I, p. 194.

Castellani and Chalmers (1913) [1] write that the results of experiments tend to show that temperature has most effect upon the ookinete before it pierces the wall of the stomach of the mosquito and becomes encysted; and that it would appear that if the temperature is below 15° to 16° C. no further development of the oocyst will take place in any form.

Hindle (1914) [2] writes, "At lower temperatures the development is very much prolonged, and consequently the mosquito does not become infective until after a much longer incubation period."

Walker and Barber (1914) [3] in the Philippines found that during the warm season *Anopheles* could be infected with sporozoites of *P. falciparum* in 12 days, while in the cool season sporozoites appeared to require from 13 to 15 days to develop.

Grassi [4] was probably the first among the early investigators of the etiology of malaria to appreciate the correlation of the influence of temperature on the development of the exogenous cycle with the seasonal variation of endemic malaria. The epidemiological significance of temperature relations was brought early to his notice when he attempted unsuccessfully to produce infection in mosquitoes held at 14° to 15° C. in the first hours after biting. Infection resulted under the same conditions at a temperature of 20° to 22° C. Following the extension of these observations, in a series of carefully controlled experiments, Grassi came to the following conclusions:

1. The development of the tertian and subtertian parasites can not be produced at temperatures varying from 15.5° to 17.5° C., but after the development of the parasites in the insect's midgut has begun, the temperature can without jeopardy be lowered to 9° to 11° C.

2. The tertian parasite in the *Anopheles* will develop at a temperature at which subtertian crescents will not develop.

3. The important epidemiological factor of the effect of low temperature during the first hours after biting is ascribed to the fact that exflagellation and fertilization are not produced at these low temperatures. After these phenomena take place and the ookinete is formed mature development ensues, even in the presence of low temperatures.

4. The minimum temperature for exflagellation of crescents was established at 17° C., although at this temperature exflagellation is by no means frequent. At 18° to 20° C. exflagellation is certain.

[1] Castellani, A., and Chalmers, A. J. (1913). Manual of tropical medicine. Wm. Wood & Co., New York, p. 855.

[2] Hindle, E. (1914). Flies and disease. The blood sucking flies. Cambridge University Press, Cambridge, England.

[3] Walker, E. L., and Barber, M. A. (1914). Malaria in the Philippines. The Philippine Journal of Science, Vol. IX, No. 5, sec. B., September, 1914.

[4] Grasssi, B. (1901). Die malaria. Studien eines zoologen. Jena. Quoted from Jansco.

5. The minimum temperatures for development of the various sporogonic types of parasites were established as 16.5° for quartan, 17.5° for tertian, and 18° C. for subtertian.

These findings of Grassi have been confirmed by the several authors whose work is detailed in the table presented later.

Jansco (1904)[1] repeated the experiments of Grassi and coworkers with essentially different results. Grassi's conclusion that temperatures under 16° C. during the first hours after biting prevent the formation of oocysts on account of the inhibited fertilization of macrogametes is held as untenable by Jansco. The latter's experiments indicated that fertilization occurred even under 16.0° C., not under artificial conditions on a glass slide, but in the stomach of the anopheline where the blood is supposed not to cool so quickly. Experiments showed that *Anopheles* became infected with tertian and subtertian gametes when kept from the moment of biting for 24 hours at a temperature of 11° to 13° C. and then exposed to a temperature between 20° to 30° C. However, when maintained at the original low temperature, infection did not result. Jansco concluded that greater importance should be attached to that stage in which the blood is already digested and the ookinetes are in the act of penetrating the gut epithelium than to the first hours after biting.

The essential principle in the difference in results obtained by Jansco and Grassi may be ascribed to their interpretation of the *critical temperature* involved. Grassi holds the *critical temperature* to be that immediately after biting—the temperature favoring fertilization; Jansco establishes the *critical temperature* as that favoring penetration of the ookinete preliminary to cyst formation.

The following table summarizes the experiments of these investigators:

TABLE No. 1.—*Details of experiments of various authors relative to low temperature and development in the mosquito.*

Author.	Experiment No.	Type of malaria.	Initial temperature.	Time held.	Second temperature.	Time held.	Results.
			°C.		°C.		
Grassi	1	Tertian. Subtertian with *A. claviger*.	15.5–17.5	Immediately after biting.			No development.
	2	Tertian. Subtertian.	Sufficient for ookinete formation.	After ookinete formation.	11.9	Indefinite.	Developed normally.
	3	Quartan	do	do	16.5	do	Do.
	4	Subtertian	18 and above	Immediately after biting			Full development.
	5	Tertian	17.5 and above.	do			Do.
	6	Quartan	16.5 and above.	do			Do.

[1] Jansco, N. (1904). Zur Frage der Infection der *Anopheles claviger* mit Malariaparasiten bei niederer Temperatur. Centralbl. f. Bakt. Vol. XXXVI, p. 624.

TABLE No. 1.—*Details of experiments of various authors relative to low temperature and development in the mosquito*—Continued.

Author	Experiment No.	Type of malaria.	Initial temperature,	Time held.	Second temperature.	Time held.	Results.
			°C.		°C.		
Van der Scheer and Van Berlekom.	1	Tertian.....	14.5–16.5.....	.....do.....	.....	.....	Negative.
	2	.....do.....	18–21.5.....	.....do.....	.....	.....	4 out of 5 became infected.
Martirano.....	1	Subtertian..	17 and below	.....do.....	.....	Several hours.	Negative.
	2	.....do.....	18.....	.....do.....	.....	25–30 minutes.	Development observed.
	3	.....do.....	18–20.....	.....do.....	.....	20–30 minutes.	Crescents (changed to round bodies).
Schoo.....	1	Tertian.....	15.....	.....do.....	.....	12 days....	Negative.
	2	.....do.....	18.....	.....do.....	.....	18 days....	Oocysts mature.
	3	.....do.....	Sufficient for oocyst formation.	.....do.....	.....	10 days....	Do.

TABLE No. 1A.—*Details of temperature experiments of Jansco.*

Experiment No.	Type of malaria.	Initial temperature.	Time held.	Second temperature.	Time held.	Third temperature.	Time held.	Results.
		°C.		°C.		°C.		
1	Subtertian.	31	30 min	13	7 hours..	30	4 days 16½ hours.	2 of 4 mosquitoes with oocyst, size 17 μ.
2	Subtertian with *A. claviger*.	31	30 min	13	22 hours.	24	5 days 21½ hours.	2 of 6 mosquitoes with numerous oocysts 6–8 μ in size.
3	.....do.....	30	30 min	11	6 hours..	30	5 days 17½ hours.	4 of 10 mosquitoes with oocysts 22–28 μ.
4	.....do.....	30	30 min	11	8 hours..	30	5 days 15½ hours.	1 of 6 mosquitoes found infected.
5	.....do.....	11	8 hours	30	10 days..	.....	.....	1 with oocysts to size of 44 μ.
6	.....do.....	11–13	10 days	.....	.....	.....	.....	None infected (13 used).
7	.....do.....	30	10 days	.....	.....	.....	.....	8 of 12 infected.
8	.....do.....	13	2 hours	22	4 days...	.....	.....	1 of 5 infected with oocysts 6–9 μ.
9	.....do.....	30	4 days.	.....	.....	.....	.....	1 of 8 infected.
10	.....do.....	13	22 hrs .	22	24 hours.	[1] 22	9 days 14 hours.	2 of 4 mosquitoes infected—1 with 1 oocyst (sporoblast), 1 with 12 oocysts, size 30 μ.
11	.....do.....	30	2 days.	8	5 days...	30	5 days.....	5—all negative.
12	.....do.....	30	12 days	.....	.....	.....	.....	3 of 7 infected.
13	.....do.....	21	4 days.	8	4 days...	20	3 days.....	1 with 40–50 oocysts 7–9 μ in size.
14	.....do.....	.....	.....	.....	.....	[2] 8	5 days.....	Several infected with oocysts up to 11 μ in size; some ookinete found unchanged.
15	Tertian with *A. claviger*.	11	22 hrs.	21	5 days...	.....	.....	The single mosquito used found with 6 oocysts 7 μ in size.
16	.....do.....	13	4 days.	22	10 days..	.....	.....	7 negative.
17	.....do.....	13	14 days	.....	.....	.....	.....	9 negative.
18	.....do.....	20	14 days	.....	.....	.....	.....	26 out of 40 infected.

[1] After holding 12 hours at 10°.

[2] After holding 3 days at 20°.

In analyzing the results of temperature experiments, it is apparent that no cognizance is taken of the lack of sporozoite development either in oocysts or in gland cells at low temperatures. This may be

due to the fact that the infected mosquitoes have not been kept alive long enough to permit the development of mature forms, or the workers may have assumed that the presence of oocysts up to the development of sporoblasts was sufficient evidence of the infectibility of the mosquito.

In this connection, the epidemiological application of the experiments of Jansco and Grassi is open to question, as the exposure of anopheline mosquitoes to a change of temperature of 8 to 22° C. immediately after engorgement could not be expected to occur under natural conditions.

The writer has attempted to extend the low temperature experiments of previous writers in order to determine what changes occur in the malarial parasite within the body of the mosquito during the period of hibernation under natural conditions. Mosquitoes were kept at living room temperature during 10 to 13 days following the initial gametocyte bearing blood meal. They were then subjected gradually to outdoor winter temperature (New Orleans) in a large cage protected from wind and rain. They were removed from time to time in order to permit them to obtain a blood meal from rabbits kept for this purpose.

The specimens were laboratory-bred anophelines kept individually in commodious lantern chimneys fastened at both ends with bobinet held by tape and rubber bands. Moisture was provided in the gauze pad in a tray in which four of the cages were placed on end and raisins were placed on the upper ends during the intervals of blood feeding. As many as three bites were taken by each mosquito when applied to the patient and subsequently a rabbit was employed to supply blood to increase the longevity of the mosquitoes. It was observed that blood was taken more readily when the raisin diet was withheld.

A gradual adaptation to low temperature was attempted by the following precautions:

The mosquitoes after biting were kept in the room with the patient for several hours, then transferred to a living room for the period stated during which time the temperature was maintained at 20° to 26° C. At this time the outdoor temperature being much lower, the specimens were retained for several days in the low-temperature incubator regulated through the use of ice and electricity, at 15° to 18° C. Then the outdoor cage was used, the temperature being recorded as shown in table No. 2.

The following table represents the temperature and humidity to which the mosquitoes were exposed in the outdoor cage during the period of the experiment, November 1 to January 11.

TABLE NO. 2.—*Record of daily temperatures and average relative humidity Nov. 1, 1916, to Jan. 15, 1917.*

Date.	November.				December.				January.			
	Maximum.	Minimum.	Mean.	Humidity.	Maximum.	Minimum.	Mean.	Humidity.	Maximum.	Minimum.	Mean.	Humidity.
	°C.	°C.	°C.		°C.	°C.	°C.		°C.	°C.	°C.	
1	27	19	23	64.5	21	9.5	15.5	70	25	18	21.5	88
2	28.5	17.5	23	48	23	10.5	16.5	80.5	27	10	18.5	84.7
3	28	18	23	54.5	24	15	19.5	84.5	28	20.5	24.2	78
4	28.5	19	23	76	28	19	23.5	84.5	29	21	25	81
5	28	19	23	84.5	27	19	23	87.5	20	17	18.5	65.3
6	27	17	22	91.5	26.5	18	22	96.5	17	11	14	51
7	28	19.5	24	89	26	19.5	23	95.5	18.5	12	15.2	58.7
8	29.5	20.5	25	89	25	9	17	88.5	22	13.5	17.2	59
9	28	19	23.5	88.5	11.5	5	8	63	25.5	16.5	21	78
10	21	18	20.5	84.5	15.5	6.5	10.5	79	27	21	24	60
11	27	18	23	85.5	16	9	13	75.5	13	9	11	43.7
12	28.5	19.5	24	95	11.5	3	7	63.5	18	7.5	13.2	66.3
13	28.5	21	25	93.5	16.5	7	12	74	24	16.5	20.2	82.7
14	22.5	6	14.5	77	22.5	10.5	16.5	85.5	11	3.5	7.2	69.3
15	12	3	7	52	10.5	2.5	6.5	75.5	11	7	9	93.3
16	13.5	4	8.5	55.5	17	5	10.5	67.5	.....	.....	.....	.....
17	20.5	7	13.5	75	19.5	11.5	15.5	97	.....	.....	.....	.....
18	18	10.5	14	56	20.5	6.5	13	71	.....	.....	.....	.....
19	22	10.5	16.5	78	15.5	4	9.5	83	.....	.....	.....	.....
20	25	13	18	83	28.5	16	22	91.5	.....	.....	.....	.....
21	27	16	21	86	24	6	15.5	89	.....	.....	.....	.....
22	27.5	17.5	22.5	87	10.5	2.5	6.5	71.5	.....	.....	.....	.....
23	19.5	13	16.5	72.5	17	5	10.5	93	.....	.....	.....	.....
24	17.5	10.5	14.5	61.5	22.5	13	17.5	97	.....	.....	.....	.....
25	15	7	10.5	64	22	15.5	18	99.5	.....	.....	.....	.....
26	17	7	12	72.5	26.5	19	23	94.5	.....	.....	.....	.....
27	19	13	15.5	87	27	23.5	25	94	.....	.....	.....	.....
28	27	16	21	93	24.5	20.5	22.5	94.5	.....	.....	.....	.....
29	25	15.5	19.5	95.5	20.5	17	18	96.5	.....	.....	.....	.....
30	21	15	18	84	20.5	15	17.5	87	.....	.....	.....	.....
31	.....	.....	.....	.....	19.5	16.5	18	97	.....	.....	.....	.....
Monthly averages	24	14.5	18.5	77.5	20.5	11.5	16	84.7	21	14.9	17.3	70.6

In order to determine the effect of transferring to much higher temperatures, at the close of the experiment 8 specimens were removed from the outside cage and placed in the room incubator, which, during the two weeks of the test, registered 20° to 32° C., with a mean temperature of 24.6° C. The mosquitoes were dissected at intervals with the following results: Two of the 8 specimens were found infected. One of the infected mosquitoes was found with 2 empty oocysts shrunken and ruptured and containing only granules of residual protoplasm. The glands proved negative. The infection of the other specimen was represented by empty oocyst capsules, apparently full sized, devoid of contents except for a few sporoblast-like bodies in one oocyst. The glands were not infected in this specimen.

The results of subjecting mosquitoes to low temperatures after feeding are detailed in the following table:

TABLE No. 3.

Specimen No.	Number of bites.	Date of dissection.	Days of development.	Result.	Specimen No.	Number of bites.	Date of dissection.	Days of development.	Result.
1	2	Nov. 15	13	Positive.	28	1	Dec. 9	30	Negative.
2	2	Nov. 17	15	Negative.	29	3	Dec. 10	38	Do.
3	2	...do...	15	Do.	30	3	Dec. 12	40	Do.
4	2	Nov. 18	15	Positive.	31	3	...do...	40	Do.
5	3	...do...	18	Negative.	32	1	Dec. 14	35	Do.
6	2	...do...	16	Do.	33	3	Dec. 17	46	Do.
7	3	Nov. 19	17	Positive.	34	1	Dec. 21	34	Do.
8	3	Nov. 20	18	Negative.	35	3	Dec. 22	52	Do.
9	3	...do...	20	Positive.	36	2	Dec. 24	53	Do.
10	1	Nov. 22	10	Negative.	37	1	...do...	37	Positive.
11	1	...do...	5	Do.	38	3	Dec. 25	53	Do.
12	2	...do...	20	Do.	39	1	Dec. 26	47	Do.
13	3	Nov. 23	23	Do.	40	1	Dec. 28	41	Negative.
14	1	Nov. 25	8	Do.	41	3	Dec. 29	57	Positive.
15	3	...do...	25	Positive.	42	3	Dec. 30	58	Negative.
16	3	Nov. 28	26	Negative.	43	1	Dec. 31	44	Do.
17	2	Nov. 29	27	Do.	44	2	Jan. 1	59	Positive.
18	3	Dec. 1	29	Do.	45	1	...do...	45	Negative.
19	2	Dec. 2	30	Do.	46	1	Jan. 3	47	Positive.
20	3	Dec. 3	31	Positive.	47	1	Jan. 4	48	Negative.
21	3	...do...	31	Do.	48	2	...do...	62	Do.
22	3	...do...	33	Do.	49	1	...do...	48	Positive.
23	3	Dec. 4	32	Negative.	50	1	Jan. 5	49	Negative.
24	3	...do...	32	Do.	51	3	Jan. 11	70	Do.
25	2	Dec. 5	33	Do.	52	2	...do...	69	Do.
26	2	Dec. 8	35	Do.	53	2	...do...	70	Do.
27	3	...do...	36	Do.	54	2	...do...	70	Do.

A total of 15 infections resulted among 54 specimens of *Anopheles punctipennis* fed 10 to 70 days previously on blood containing many subtertian gametocytes. The resulting infections are described in the following table:

TABLE No. 4.

Date of dissection.	Days of development.	Stage of development.
Nov. 15	13	8 oocysts without protoplasmic differentiation, size approximately 25μ to 28μ.
Nov. 18	15	Approximately 250 oocysts in all stages preceding the sporoblastic.
Nov. 19	17	8 oocysts, 2 of which still retaining pigment, remainder granular without sporoblasts.
Nov. 20	19	Approximately 200 oocysts in all stages up to sporoblastic.
Nov. 25	24	143 oocysts, size 25μ to 40μ mostly with malarial pigment, few with sporoblasts.
Dec. 3	31	About 120 oocysts, half of them retaining pigment, only one with sporoblasts.
Do.	31	Approximately 250 oocysts, of which 50 were quite small (15μ to 20μ), with malarial pigment, remainder various sizes but more matured. Few with sporoblasts.
Do.	32	30 oocysts in various stages, a few with immature sporoblasts.
Dec. 24	37	One oocyst represented by shrunken capsule without contents, oocyst apparently full sized and firmly attached to gut wall.
Dec. 25	53	5 oocysts—4 with contents expelled, 1 with sporoblast development barely commencing (only 4 segments discernible). Remainder of body of oocyst undifferentiated and granular. Size 30μ by 33μ.
Dec. 26	47	3 oocysts with contents ruptured, all torn from gut wall during dissection. No evidence of sporozoites in mounting liquid surrounding the gut wall or in the glands.
Dec. 29	57	2 ruptured shrunken oocyst membranes on posterior end of midgut. No indications of sporoblasts or sporozoites.
1917.		
Jan. 1	59	1 oocyst 22μ to 25μ containing granules only. Also 4 ruptured oocyst capsules still attached to stomach wall, no sporozoites present.
Jan. 3	47	23 oocysts, the majority of which were large, size up to 65μ; 2 bodies still retained small amount of pigment; 1 very small (about 20μ). Remaining 21 oocysts of the usual sort with undifferentiated protoplasm except that 3 of them were developed to sporoblast stage. Not any of them contained sporozoites. One empty shrunken capsule was seen. Midlobe of each gland parasitized with a moderate number of sporozoite-like filaments (nonmotile) and did not stain with Giemsa.
Jan. 4	48	Infection represented only by two empty oocyst shells attached to gut wall. No sporozoites on gut or in glands.

The eight control specimens of *A. quadrimaculatus* yielded 4 infections as follows: One specimen of *A. quadrimaculatus* which proved infected was examined on the 12th day after biting the blood donor. The gut wall was covered by probably at least 200 oocysts. These were not over 35μ in size, the majority exhibiting malarial pigment and averaging 20μ to 25μ in size. No mature oocysts were seen, and the glands were devoid of sporozoites.

The second control *A. quadrimaculatus* found infected was examined 40 days after its bite of the blood donor. On the gut wall of this specimen were seen 3 oocysts and 3 shrunken capsules devoid of sporozoites or other contents. The oocysts measured 59μ to 67μ in size with undifferentiated granules lacking evidence of sporoblast development. A prolonged search was made of the mounting fluid surrounding the gut wall but sporozoites were not found. The six lobes of the salivary glands were likewise uninfected.

Another specimen of *A. quadrimaculatus* was found infected on the 40th day of development. Here were seen three empty oocyst capsules and three large oocysts, one of which measured 59μ by 65μ and the other two were as much as 67μ in diameter. The development of these oocysts was apparently abortive as sporoblasts were absent and sporozoites were not present in the mounting fluid about the stomach wall or in the six gland lobes.

The fourth specimen of this species found to be infected was dissected 54 days after its initial blood meal. The only indication of its infection was the presence of two apparently full-sized oocyst envelopes devoid of contents except for a few residual sporoblast-like bodies in one of them. The glands were negative except for a moderate invasion of sporozoites in the midlobe of one gland.

The single specimen of *A. quadrimaculatus* in which sporozoite development was demonstrated had been kept at room temperature (gas heated, mean temperature of approximately 22° C.). The other three specimens were subjected to the same conditions of temperature and humidity as the specimens of *A. punctipennis*.

The mosquitoes employed in these experiments were allowed a maximum period of 70 days in which to produce gland sporozoites. Only one of the series kept at low temperatures showed bodies which resembled sporozoites, but because of their peculiar character and unusual behavior their identity is questionable. These bodies, found in a specimen of *A. punctipennis* after an interval of 47 days following a single infective bite, were of the usual filamentous type, of normal size but with no appearance of nuclei. Only the two mid lobes of the glands contained a moderate number of the filaments. None of the oocysts invading the stomach wall contained filaments, although sporoblasts were seen in 3 of the 23 oocysts, the majority of which were of mature size. The salivary glands of this mosquito

were given a prolonged study, but no evidence of the characteristic writhing movement or other sign of viability was observed in the spindle forms present. Slight warming to 30° C. caused no change in the material placed in normal saline, and when the gland cells were ruptured by pressure and macerated, no activity followed. When stained with Giemsa solution, the bodies smeared from the glands did not take the stain so as to be recognizable.

The contents of the salivary glands of two other specimens were also suspected on account of the presence of sporozoite-like bodies, but in these instances one could feel fairly confident that they were only the peculiar threadlike crystals described by Stephens as artifacts.

Factors Other Than Temperature Influencing Parasitism.

It has been shown that other factors besides temperature may influence infectivity, but aside from the degree of parasitism in the human host and the number of gametocytes ingested by the mosquito, little is known.

Daniels (1901) [1] emphasizes the fact that infection is directly dependent on the number of bites the mosquito takes from the patient and has shown that the infection varies from 26 per cent to 66 per cent, depending on the number of times the mosquitoes were permitted to bite the gametocyte carrier. He reported 27 infected mosquitoes of 57 applied, distributed as follows:

Number of bites.	Percentage infected.
1	26
2	46
3	62
4	66

In the work presented here analogous results were obtained. The percentage of infections was proportional to the number of bites the insects took. In the following table 19 mosquitoes of two species are accounted for in relation to the number of infective bites obtained from the human host, a subtertian case:

TABLE No. 5.

	Number of times fed.	Number of mosquitoes.	Number positive.	Percentage positive.
A. punctipennis	1	32	4	12.5
Do	2	16	3	18.8
Do	3	23	8	34.8
A. quadrimaculatus	1	5	1	20.0
Do	2	4	2	50.0
Do	3	1	1	.....

[1] Daniels, C. W. (1901), Malaria. British Medical Journal. Jan. 26. Cited by Deadrick, W. H. (1911), A practical study of malaria. Saunders Co., Philadelphia and London. p. 70.

The elimination of gametes through digestive activity of the mosquito may be considered another factor, relative especially to the loss of infection in certain *Anopheles*, and is discussed because it has been ignored or overlooked heretofore.

Darling (1910) [1] has ingeniously accounted for the failure of infection in the mosquito host to the extent of 97 per cent mainly through the phenomenon of phagocytosis. He concludes that the gametocytes accruing from three successive blood meals are retained by the insect and that the fertilized gametes, if they do not become phagocytized have abundant time to wander out of the blood clot and reach the gut wall.

In addition to this, one must take into account the peculiar habit of the mosquito to "clear" itself, by discharging blood per anum during and for some time following a blood meal. It should be considered as an important source of gametocyte elimination. This means of limiting the number of zygotes is demonstrated in the finding of as many as 12 crescents in a single field of the bloody dejecta. This process takes place at the time most opportune—before fertilization and subsequent encystment of parasites. The early excretions—during perhaps the first 24 hours—are the most important in this regard, as the clearing process is a mechanical one and the phenomenon of exflagellation does not get an opportunity to establish itself. To be sure, this elimination process requires 1 to 5 days or more for completion, depending on the temperature. Later defecations are associated with normal degenerative changes, so that this elimination is not significant after the early hours of biting.

In a study of the contents of excreted blood numerous crescents have been encountered possibly just as relatively abundant as, or more so than, those contained in the peripheral blood of the human host. The forms seen are similar to those in the patient's blood except that in addition to deformed crescents, many fragmentary bodies suggestive of active phagocytosis or changes due to insect alimentation are commonly seen. A series of counts made of stained films of this excreted material, taken from 10 to 30 minutes after the mosquitoes had bitten, indicated that the crescents were somewhat concentrated. There were present 87 crescents to each 100 leucocytes, while in the blood film taken previously to the biting, 63 crescents per 100 leucocytes were counted.

A true valuation of this observation could be obtained only in careful weighings of mosquitoes at various stages after biting, and in blood counts and estimations such as Darling has employed in his studies.

Concentration of the gametes in the dejecta, if it does occur, may be tentatively explained by the rise of these bodies in the same way

[1] Darling, S. T. (1910), Studies in relation to malaria. Bulletin Isthmian Canal Commission Press.

that the crescents rise when a tube of infected blood is centrifuged in Bass and Johns' (1915) [1] method of concentrating the parasites for diagnostic and cultural purposes. These workers discovered that when blood containing crescents was centrifuged the parasites rose to the top of the cell column so that a mass of almost pure crescents could be thus obtained. The alimentary canal of the mosquito may be compared to a centrifuged tube in which the blood is agitated through the processes of biting and subsequent peristalsis. It is suggested that the action may be aided by the raising of the caudal end of the abdomen which is done by the anopheline in biting and resting.

This theory may be further strengthened by the fact, observed by all investigators of the mosquito cycle, that there is a decided concentration of oocysts on the gut wall toward the anal end. Especially is this the case when only few oocysts are present. We may presume that the developed bodies appear in this location on account of the presence of the greatest number of gametes at the distal end of the gut.

Interpretation of Results and Summary.

In the work presented here it is indicated that development of the exogenous elements in the mosquito is restricted or prevented during an intermittent low temperature even when temperatures favorable to parasite development are present in the early stages and subsequently.

That the presence of even great numbers of oocysts in various stages does not give assurance of subsequent maturity and infectivity is evidenced in these experiments.

Of the 18 infected *Anophelines* kept at low temperature only one appeared to give rise to mature parasites, while the one control specimen of *A. quadrimaculatus* retained at room temperature reached normal maturity relative to sporozoite development. *Plasmodium falciparum* was the species of parasite used.

The oocyst stage was maintained up to 59 days in the mosquitoes employed in these experiments. A peculiar appearance of these bodies gave the impression that development would not be carried to maturity even if at this period mosquitoes were exposed to salubrious temperatures. Sporozoites were not produced in eight mosquitoes of this series which had been exposed as much as 60 days to intermittent low temperature, then transferred to an optimum temperature for two weeks longer. Two of the eight mosquitoes proved to be infected by only a variable number of shrunken and ruptured oocyst capsules.

[1] Bass, C. C., and Johns, F. M. (1915). A method of concentrating malaria plasmodia for diagnostic and other purposes. Am. Jour. of Trop. Dis. and Prev. Med., Vol. III, No. 5, November, pp. 298-303.

A suggestion of the mode of evolution in the growth and subsequent degeneration of the bodies found in the mosquitoes may be given as follows:

The nature of the oocyst throughout the incubation period was such as to indicate that development was practically negligible after about 19 days and up to 59 days. Taking as an illustration the development produced in a mosquito during 47 days of incubation, we find bodies indistinguishable in morphology and size from similar bodies seen in mosquitoes during 13 to 19 days of development.

Even up to 31 days the presence of malarial pigment could be demonstrated in numerous oocysts. This of course may be interpreted as aborted development brought about by low temperatures. Also up to this time (31 days) few sporoblasts were seen among the oocysts encountered, possibly another influence of low temperature. Beginning with the thirty-seventh day it was found that oocysts commenced to degenerate, rupturing prior to sporozoite development. Numerous ruptured oocysts were seen up to the fifty-ninth day, and not in a single instance was the presence of sporozoites revealed. During this interval many oocyst capsules were found unattached to the gut wall, probably having been dislodged in the process of dissection.

The absence of sporozoites, with one exception, in the 18 specimens infected is significant. In the one exception it is to be noted that the presence of sporozoites is open to question on account of the uncharacteristic form and behavior of the bodies seen.

The loss of infectivity through temperature change is significant in relation to hibernation of infected mosquitoes. Much can be explained if it should be definitely proved that low temperature prevents sporozoite development in mosquitoes inactive during the winter.

It is indicated in the results of these experiments that an intermittent low temperature does interfere with sporozoite formation; consequently it is explicable that mosquitoes procuring gametocyte bearing blood before winter sets in, may become sterile or innocuous during the hibernation period.

The writer has obtained a partial confirmation of the results of Daniels in the relation of infection to the number of bites which the mosquitoes obtain. Fifteen examples of *A. punctipennis* gave the following results relative to infection with the parasites of malaria: One, two and three bites gave 12.5, 18.8 and 34.8 per cent respectively. The results obtained with 4 specimens of *A. quadrimaculatus* were 20, 50, and 100 per cent relative to one, two, and three bites obtained.

Another factor besides low temperature which possibly influences infectivity was found to be the loss of gametocytes through the

"clearing process" in the mosquito. This is indicated in the blood count of the mosquitoes' dejecta, in which numerous crescents were found. In one instance blood from the human host yielded 63 crescents to 100 leucocytes and in the blood after passing through the mosquito 87 crescents to 100 leucocytes were counted.

Addendum.

In a recent paper King (1917),[1] working in New Orleans, has shown some interesting results relative to low temperature influence on the sporogonic development. He shows that the parasite of tertian malaria in *Anopheles quadrimaculatus* is able to survive exposure to a temperature of 30° F. for a period of 2 days, 31° F. for 4 days, 45° to 69° F. for 6 to 7 days, and in two mosquitoes 38° to 59° F. for 17 days. In a smaller series of tests the sporonts of *P. falciparum* showed a resistance to 35° to 57° F. for 1 to 2 days.

In these experiments the parasites in the mosquito were permitted to develop during 7 to 23 days at room temperature before the insects were exposed to temperatures of 29° to 69° F. for periods ranging from 1 to 16 days, following which they were maintained at room temperature for an additional period of 1 to 19 days.

It is indicated from these tests that exposure to low temperatures, for a limited period at least, did not affect the viability of sporozoites assuming that provision had been made for the mosquitoes to develop sporozoites at room temperature.

[1] King, W. V. (1917), The effect of cold upon malaria parasites in the mosquito host. The Journal of Experimental Medicine, Vol. XXV. No. 3, March, pp. 495-498.